DIVISION S-3—SOIL BIOLOGY & BIOCHEMISTRY

Carbon and Nitrogen Dynamics During Incubation of Manured Soil

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ABSTRACT

Denitrification N losses during manure net mineralizable N assays may lead to miscalculation of the manure's N-supplying capacity. In this study we measured denitrification, manure properties, gas fluxes, nutrient pools, and mineralizable N during laboratory incubation of manured soil. Different dairy manures (n = 107) were added to soil at a rate of 0.1 mg N g^{-1}. Manured and control soils were incubated and sampled weekly for soil mineral N, CO_2 flux, and N_2O flux. The denitrification enzyme activity (DEA) was measured at the end of the experiment. Weekly N_2O and CO_2 production increased in the manured soils during the first 3 wk of incubation. There was a positive correlation between added manure C and cumulative CO_2 production. Nitrate content increased in all soils throughout the 6-wk period, but the increase was more marked in the manured soils. In most manured soils, ammonium concentration was initially high then declined rapidly during the first 2 wk. This high net NH_4^+ decline in the manured soils suggests that N was immobilized during the incubation. Microbial biomass N should be determined during manure mineralization assays to account for all potential manure N sinks. No correlation existed between DEA and N pools or gas fluxes in the manured soils. Manures with negative N mineralization had an average C/N of 19.0, while manures with positive N mineralization had an average C/N of 16.0. On average, denitrification accounted for approximately 5% of the added manure N. Higher proportions of denitrified N were observed in some manures, supporting our hypothesis that N losses through denitrification may be significant in manure mineralizable N assays.

The quality of a manure as an N fertilizer is difficult to predict, since manure composition may vary widely according to diet and other management factors (Reeves and Van Kessel, 2002; Van Kessel and Reeves, 2002). Dairy manure is a complex mixture of materials with varied mineralization kinetics ranging from relatively resistant lignin to readily available ammonium and volatile fatty acids (VFA) (Van Kessel et al., 2000).

Laboratory incubations are performed to determine the N mineralization potential of manure-amended soils (Bernal and Kirchmann, 1992). Incubation studies have shown that the mineralization potential of manures may be related to manure C/N ratio and manure N content (Jedidi et al., 1995). According to incubation studies, some manures act as net suppliers of N, while others may result in net N immobilization. Hadas and Portnoy (1994) determined that manures might release up to 29% of their N content as inorganic N during a laboratory incubation. In contrast, Sørensen (1998) found net immobilization of N during manure incubations, and the effect was pronounced with manures rich in VFA content. In addition, N losses through denitrification as well as N immobilization during laboratory incubations may affect the correlation of incubation data with manure N mineralization in the field, as well as preventing a good agreement between manure N pools and mineralizable N.

We hypothesize that denitrification may divert manure mineralizable N to N gas, resulting in inaccurate estimation of mineralizable N during laboratory incubations. Because of this, accounting for denitrified N may improve the correlation between manure N and mineralizable N. Farmers and extension agents need accurate information regarding manure N availability to make sound manure management decisions. Previous work to determine denitrification N losses after manure application has included few manures per study. However, to better represent field variability, it is necessary to examine and compare a large number of manures. In this study, we collected manures from a wide variety of farms each with different storage conditions, resulting in a set of manures that varied widely in composition.

The objectives of this experiment were to: (i) determine the effect of manure addition on soil C and N dynamics; (ii) determine if manure chemical or nutritional properties correlate to C and N pools, gas fluxes or denitrification during a laboratory incubation of manured soil; and (iii) measure the amount of N lost through denitrification during a mineralizable N assay. To achieve these objectives, we performed 6-wk laboratory incubations of manured soils. Soil mineral N, CO_2 flux, and N_2O flux after acetylene addition were measured weekly. Manure properties were measured before the incubation experiment, while DEA was measured at the end of the 6-wk incubation.

MATERIALS AND METHODS

Soil and Manure Collection

The Christiana fine sandy loam soil (typic Normudults) used in this study was obtained from an alfalfa field located on the USDA-ARS Beltsville Agricultural Research Center. The soil was obtained from the Ap horizon and had an organic C of 19.3 mg g^{-1}, and total N content of 1.6 mg g^{-1}. Clay, silt,

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Published in Soil Sci. Soc. Am. J. 68:1592–1599 (2004).

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Abbreviations: ADF, acid detergent fiber; DEA, denitrification enzyme activity; FDM, dry matter fiber; NDF, neutral detergent fiber; VFA, volatile fatty acids.

and sand contents were 11, 18, and 71%, respectively. Before the experiment, the soil was sifted (1 cm) to exclude coarse rocks and plant debris.

Dairy manures (n = 107) were collected during the fall of 1998 from farms in the eastern USA (CT, MD, NY, PA, and VA). All samples were stored at −20°C from 1998 until the start of the experiment in the summer of 2001. It has been shown that freezing manure is an adequate storage method that does not significantly affect the N mineralization properties of the manure (Van Kessel et al., 1999).

Soil Incubations

Before the experiment, the soil was allowed to dry at room temperature to achieve a soil gravimetric moisture of 15.6% before manure addition. To prepare the microcosms, 100 g dry soil (115.6 g fresh weight) was packed into 100-mL plastic beakers. Each manure was homogenized by blending with dry ice (1:2 manure/dry ice, v/v) at high speed in a Vita Mix 3600 Plus (Vita Mix Corp., Cleveland, OH). After blending, the CO_2 was allowed to evolve from the manures at 5°C. Exactly 5 mL of manure + water was added uniformly to the top of the packed soil. The volume of the manure was adjusted so that each microcosm received exactly 0.1 mg of manure N g^{-1} soil (equivalent to 265 kg N ha^{-1}). With this manure addition scheme, the microcosms received a range of manure C of 0.2 to 2.17 mg g^{-1} soil.

Two replicates from each manure treatment and 12 nonmanured controls were destructively sampled each sampling time. There were seven sampling times consisting of time zero, plus weekly samplings for 6 wk. With this design, 14 jars from each of the 107 manure microcosms were sampled throughout the experiment, and a total of 1582 manured and control microcosms were included in the experiment.

The microcosms were put in 3.8 L gas-tight jars to allow for gas flux sampling and to provide a closed system where N losses through ammonia volatilization would be minimized. The jars were incubated at 22°C, and were opened weekly to aerate the microcosms. Moisture loss from the microcosms was minimized by adding 2 mL of water to jars to maintain saturated humidity in the headspace. Average initial gravimetric moisture was 16.8% (25.5% water filled pore space), and at the end of the incubation, the average gravimetric moisture declined to 15.5% (23.1% water filled pore space).

One week before each destructive sampling, the microcosms received 40 mL of acetylene. The N_2O fluxes of jars receiving acetylene are hereafter referred to as N_2Oa. With this design, each microcosm was sampled weekly for headspace N_2O in the absence of acetylene during a period that varied from 0 to 5 wk, depending on the sampling time that the microcosm was allotted to. Weekly gas sampling of the microcosms allotted to the last destructive sampling that had not yet received acetylene (hereafter referred to as N_2On) allowed a pair wise comparison with N_2Oa jars. Immediately after the headspace gas sampling, the microcosms were taken out of the jars and the soil from each microcosm was homogenized by mixing with a spatula. Samples from the soil homogenate were then taken for the extractable soil mineral N and denitrification enzyme activity analysis (see below).

Manure Analysis

All manures were analyzed for several chemical, physical, and nutritional variables before the incubation experiment. The gravimetric moisture content of the manures ranged from 60.3 to 98.6%. Each manure was mixed and subsampled for analysis of several manure properties (Table 1). Subsamples were dried (60°C) and ground (850 μm), then sent for dry matter fiber (FDM), acid detergent fiber (ADF), neutral detergent fiber (NDF), and lignin analysis to the Dairy One DHI Forage Testing Laboratory (Ithaca, NY). The P_2O_5 and K_2O were analyzed by the University of Maryland Soil Testing Laboratory (College Park, MD). Manure N and C were measured by combustion of dry (55°C) samples using a LECO Carbon analyzer (LECO Corp., St. Joseph, MI). For mineral N analysis, manure samples (3 g fresh weight) were placed in Erlenmeyer flasks with 300 mL of 2M KCl. The flasks were stoppered and shaken for 30 min. The extracts were allowed to settle overnight at 5°C. After settling, supernatant was pipetted to a 20-mL scintillation vial and stored at 5°C until analysis. The nitrate and ammonium concentration in the extracts was measured colorimetrically using Lachat Flow Injection Analyzer (Lachat Instruments, Milwaukee, WI).

For the VFA analysis, manure samples (100 g) were mixed with 100 mL of H_2O in a 500-mL Erlenmeyer flask and mixed every 10 min. for 1 h. After the solids settled, 50 mL of supernatant was centrifuged (40 min., 18 400 × g; 11 000 rpm). The supernatant was transferred and frozen until analysis. Five milliliters of thawed extract was acidified with 75 μL of concentrated sulfuric acid and centrifuged (15 min., 18 400 × g; 11 000 rpm). One milliliter of this supernatant was mixed with 0.4 mL of 2.004 g L^{-1} 2-ethylbutyric acid, as an internal standard. This solution was analyzed for VFA with a Hewlett-Packard 5890 series II gas chromatograph fitted with a flame ionization detector and a column (1.83 m, 2 mm diam.) packed with 10% SP-1200 and 1% H_3PO_4 on Chromosorb W/AW 80/100 mesh. The injector was held at 200°C, and the detector temperature was 220°C. The column temperature was isothermal at 120°C.

Headspace Analysis

At each sampling time, CO_2 flux was measured immediately before each of the microcosms were destructively sampled. Two milliliters of the jar headspace were injected into 22-mL vials fitted with butyl rubber septa and previously flushed with He. The CO_2 content was analyzed using a Tekmar 7000 HT

Table 1. Average and range values of the manure properties analyzed before the experiment. n = 107.

Variable	Units	Average (SEM)	Range
pH		7.1 (0.0)	6.1–8.0
N	g g^{-1} dry weight × 100	2.3 (0.1)	0.9–3.3
C	g g^{-1} dry weight × 100	41.6 (0. 6)	14.0–76.1
C/N		18.7 (0.4)	11.3–38.9
Dry matter	g g^{-1} fresh weight × 100	0.1 (0.0)	0.0–0.4
Ammonium	mg g^{-1} dry manure	15.1 (0.7)	1.7–39.5
Dry matter fiber (FDM)	% dry matter basis	90.8 (0.1)	85.9–97.2
Acid detergent fiber (ADF)	% dry matter basis	40.6 (0.6)	10.6–78.1
Neutral detergent fiber (NDF)	% dry matter basis	55.6 (0.7)	17.2–82.8
Lignin	% dry matter basis	10.5 (0.2)	2.0–18.2
P_2O_5	% fresh weight	0.34 (0.13)	0.03–0.64
K_2O	% fresh weight	0.20 (0.10)	0.04–0.86

headspace autosampler (Tekmar Co., Cincinnati, OH) and a Tremetrics Model 540 gas chromatograph (Tremetrics Inc., Austin, TX), using the conditions detailed in McCarty and Blicher-Mathiesen (1996). Gas samples for N_2O analysis were obtained in the same fashion and at the same time as the CO_2 samples. The N_2O analysis was performed with a Tekmar 7000 HT headspace autosampler (Tekmar Co., Cincinnati, OH) in series with a Shimadzu GC-8A (Shimadzu Scientific Instruments, Inc., Columbia, MD) fitted with an ECD detector.

Soil Mineral Nitrogen

Soil samples (10 g fresh weight) from each microcosm were analyzed for extractable soil mineral N using the same procedure and instruments as for the manure samples (see above). A ratio of 10 g of soil to 50 mL of 2*M* KCL solution was used for the extraction. In this study, the term net N mineralization is used as the change in the soil inorganic N over the 6-wk incubation (Hart et al., 1994). Previous work has referred to negative N mineralization as N immobilization, even when microbial biomass N was not measured directly (Kirchmann and Lundvall, 1993). We will instead refer to negative net N mineralization as apparent N immobilization, since denitrification N losses often do not account for the decline in soil mineral N during the incubation.

Denitrification Enzyme Activity

Duplicate soil samples (four per manure) were analyzed from the Week 6 microcosms using a modified procedure for DEA (Tiedje, 1994). Soil samples (20 g fresh weight) were placed in 100 mL serum bottles and 20 mL of a slurry solution (0.4 g KNO_3, 5.0 g glucose, 1.0 g chloramphenicol, in 1 L H_2O) were added to each serum bottle. The bottles with the soils and slurry mixtures were then capped with butyl rubber septa and crimp caps. Anaerobic conditions were created by alternatively applying vacuum pressure and purging with He gas. Three milliliters of C_2H_2 were then added to each bottle followed by vigorous shaking (1 min.). The serum bottles were then placed in a shaker (200 rpm). The headspace gas was sampled at 1 and 3 h. The N_2O gas samples (1 mL) and the CO_2 gas samples (2 mL) were then stored and analyzed using the same procedure and instruments as the CO_2 and N_2O fluxes (see above).

Statistical Analysis

The PROC CORR of SAS version 8.2 (Cary, NC) was used to determine the Pearson correlation coefficients (r) among the manure properties and the incubation data, as well as the probability (p) that the correlation coefficient was different from zero. When determining the Pearson correlation coefficients, the data of the controls was analyzed separately from the data of the manured microcosms. The PROC GLM procedure of SAS was used to carry out Analysis of Variance (ANOVA) to test effects of manure addition (manured vs. control), time (week), and set. All the variables regarding the mineral N pools as well as gas fluxes during the incubation were included as dependent variables. In addition, mean separations were performed using the least significant difference (LSD) test based on a t test.

We performed multiple regressions using the PROC REG procedure of SAS version 8.2 (Cary, NC). Manure variables such as dry matter, C, total N, Organic N, P_2O_5, K_2O, NH_4–N, lignin, ADF, NDF, as well as the individual VFA were included as dependent variables. The Forward Selection (FORWARD), Stepwise (STEPWISE), Maximum R^2 improvement (MAXR), and Full Model Fitted (NONE) Model-Selection methods of the PROC REG procedure were used. By performing the different Model-Selection methods, we aimed to establish if a combination of manure properties explains a proportion of the variance of the manure mineralizable N, and also establish the relative predictive importance of the independent variables.

RESULTS

Manure Properties

Manure C/N ratios ranged widely from 11.29 to 38.88 (Table 1). As expected, C rich components such as NDF ($r = -0.73$) and ADF ($r = -0.65$) were negatively correlated with manure percentage of N (Table 2). Neutral detergent fiber was negatively correlated with manure ammonium content ($r = -0.60$; Table 2).

Seven different VFA were detected and quantified in the manure samples (Table 3). Individual VFA were correlated among themselves, with R scores ranging from 0.53 to 0.98 (data not shown). However, none of the manure VFA correlated strongly with any of the other manure properties or incubation variables.

Carbon Dioxide Fluxes

The addition of manure increased the CO_2 flux of the soils (Fig. 1). The largest difference between manured and control soils occurred at Week 1, when the manured soils had from 42 to more than 400% higher CO_2 fluxes. Cumulative C mineralization (as CO_2 flux) during the 6-wk incubations averaged 1.38 g kg^{-1} in the manured

Table 2. Correlation matrix for selected variables for all the manured soils. The control treatment was excluded from the analysis.†

	Man. % N	Man. NH_4–N	Added Man. C	ADF	NDF	Wk 0 NH4	Wk 6 NO_3	Cum. N_2Oa	Cum. N_2On	Cum. CO_2	Net N miner.	Immob.
Man. % N	1.00	0.43	−0.50	−0.65	−0.73	−0.04	0.18	−0.15	−0.07	−0.42	0.20	0.16
Man. NH_4–N	–	1.00	−0.60	−0.56	−0.60	0.51	0.43	−0.30	−0.20	−0.46	−0.27	−0.35
Added Man. C	–	–	1.00	0.46	0.62	−0.21	−0.28	0.32	0.13	0.69	0.02	0.07
ADF	–	–	–	1.00	0.87	−0.25	−0.20	0.09	0.03	0.36	0.11	0.12
NDF	–	–	–	–	1.00	−0.15	−0.21	0.21	0.10	0.52	0.00	0.04
Wk 0 NH_4	–	–	–	–	–	1.00	0.41	−0.04	0.12	0.00	−0.81	−0.72
Wk 6 NO_3	–	–	–	–	–	–	1.00	−0.14	−0.20	−0.17	−0.20	−0.29
Cum. N_2Oa	–	–	–	–	–	–	–	1.00	0.37	0.20	0.00	0.17
Cum. N_2On	–	–	–	–	–	–	–	–	1.00	0.13	−0.13	0.33
Cum. CO_2	–	–	–	–	–	–	–	–	–	1.00	−0.23	−0.16
Net N miner.	–	–	–	–	–	–	–	–	–	–	1.00	0.89
Immob.	–	–	–	–	–	–	–	–	–	–	–	1.00

† Abbreviations are as follows: Manure is man., cumulative is cum., and mineralization is miner. Apparent immobilization (Immob) is the net N mineralization minus the cumulative N_2On. Correlation coefficients above an absolute value of 0.26 have a slope significantly different from zero ($p < 0.001$). $n = 107$.

Table 3. Average and range values of the manure volatile fatty acids (VFA) analyzed before the incubation experiment. The units are mg VFA g^{-1} dry manure. $n = 107$.

VFA	Average (SEM)	Range
Acetic acid	20.1(1.4)	0.0–90.6
Propionic acid	6.6(0.6)	0.0–27.0
Isobutyric acid	0.7(0.1)	0.0–2.5
n-Butyric acid	3.0(0.3)	0.0–19.3
Isovaleric acid	0.9(0.1)	0.0–3.7
n-Valeric acid	0.6(0.1)	0.0–3.1
Hexanoic acid	0.3(0.1)	0.0–3.2
Total VFA	32.1(2.4)	0.2–139.4

soil and 0.70 g kg^{-1} in the control soils (Table 4). Cumulative CO_2 flux had a significant positive correlation with added manure C during the incubation ($r = 0.69$, $p < 0.01$; Table 2).

Soil Mineral Nitrogen

In the manured soil, ammonium was the dominant form of mineral N at the beginning of the incubation, whereas nitrate dominated at the end of the incubation (Fig. 2). Initial ammonium concentration in the manured soil was 8.7 to 47.3% higher than that of the control soil, but declined sharply during the first 2 wk of the incubation. The initial nitrate concentration was similar in the manured and control soils. Nitrate concentration was stable in both the manured and control soils during the first week of the incubation. Thereafter, nitrate increased in both soils, but the net nitrate accumulation was significantly higher for the manured soil than for the control soil. The large decrease in ammonium during the 6-wk incubation resulted in negative net N mineralization in the manured soil. Contrary to the manured soil, nitrate as well as ammonium accumulation resulted in a small, but positive net N mineralization in the control soil.

Nitrous Oxide Fluxes and Denitrification

Weekly N_2Oa and N_2On production in the manured treatment was highest in the first week of the experiment

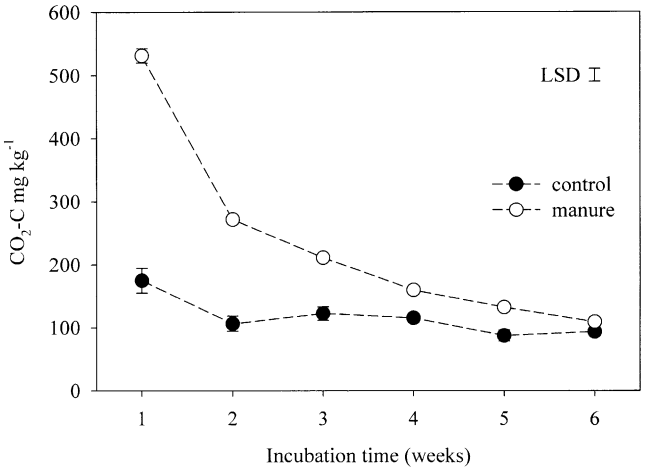

Fig. 1. Weekly CO_2 production in jars with added acetylene. $n = 107$ for the manured soil. $n = 6$ for the control soils. Error bars are the SEM. The least significant difference (LSD) according to a *t* test is shown.

Table 4. Carbon and N data of the 6-wk incubation experiment. Units for all variables are in mg kg^{-1}. Negative values indicate net consumption during the 6-wk incubation. $n = 107$ for the manured soil. $n = 6$ for the control soils. The control and manured soils were statistically different for all variables according to ANOVA ($p < 0.01$).

Variable	Average (SEM)	
	Manured soils	Control soils
Cumulative N_2O nitrogen, C_2H_4 added†	4.91 (0.39)	0.26 (0.11)
Cumulative N_2O nitrogen, no C_2H_4 added†	2.12 (1.56)	0.35 (0.11)
Cumulative CO_2–C†	1375.59 (23.60)	699.40 (35.71)
NH_4–N‡ (Net ammonification)	−10.62 (0.46)	2.25 (0.44)
NO_3–N‡ (Net nitrification)	5.68 (0.19)	2.23 (0.76)
NH_4–N + NO_3–N‡ (Net mineralization)	−4.94 (0.44)	4.48 (1.04)

† The values are the sum of the weekly values for all 6 wk.
‡ The values are the differences between the final and initial concentrations during the incubation.

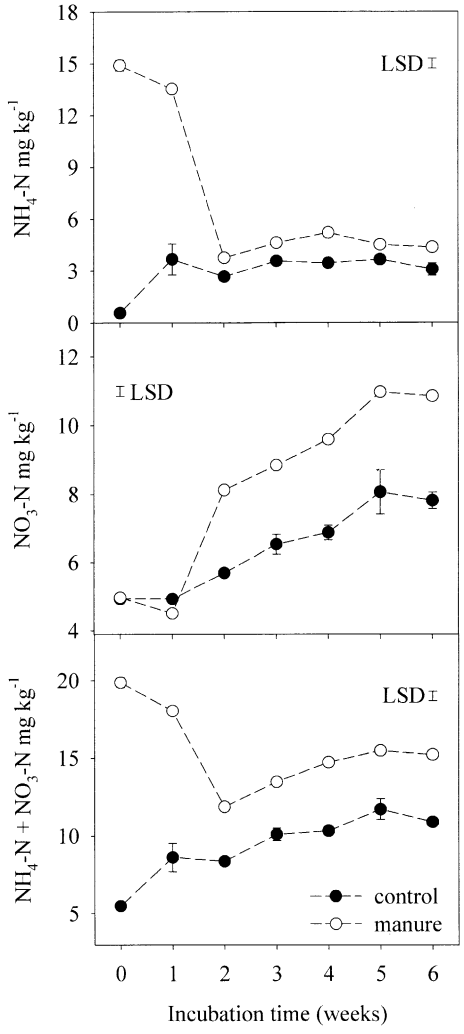

Fig. 2. Extractable mineral N pools measured during the 6-wk incubation of manured and control soils. NH_4–N (top), NO_3–N (middle), and NH_4–N + NO_3–N (bottom). $n = 107$ for the manured soil. $n = 6$ for the control soils. Error bars are the SEM. The least significant difference (LSD) according to a *t* test is shown.

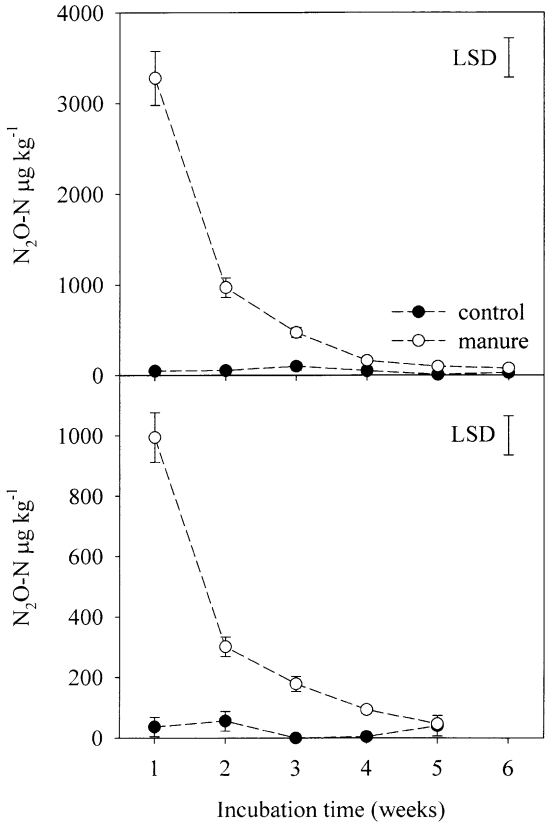

Fig. 3. Weekly nitrous oxide fluxes measured during the 6-wk incubation of manured and control soils. Acetylene added (top graph), no acetylene added (bottom graph). n = 107 for the manured soil. n = 6 for the control soils. Error bars are the SEM. The least significant difference (LSD) according to a t test is shown.

and declined afterwards (Fig. 3). The N_2Oa and N_2On fluxes in the manured soils were higher than those of the control soils during the first 3 wk of the experiment. The control soils had very low weekly N_2On fluxes throughout the incubation, regardless of the addition of acetylene. As expected, the cumulative N_2O produced during the 6-wk incubation increased when acetylene was added to manured microcosms (Fig. 3). Mean cumulative N_2Oa and N_2On in the manured soil was significantly higher than the control soils (Table 4). In the manured soils, the average N denitrified during the incubation accounts for approximately 5% of the added manure-N. However, the range of specific denitrification rates was wide, with some manures having denitrification N losses amounting to more than 10% of the added manure N (Fig. 4). In the control soils, the cumulative CO_2 flux was positively correlated with the cumulative N_2On flux (r = 0.68, $p > 0.01$).

Manure addition significantly increased the soil DEA at the end of the incubation (Table 5). In the manured soils, the DEA did not have strong correlation with any of the nutrient pools, manure properties, or gas fluxes measured during the experiment. In the control soils, DEA was positively correlated with initial soil nitrate concentration (r = 0.59). The DEA also correlated negatively with cumulative N_2On (r = −0.58), and the cumulative CO_2 flux (r = −0.63).

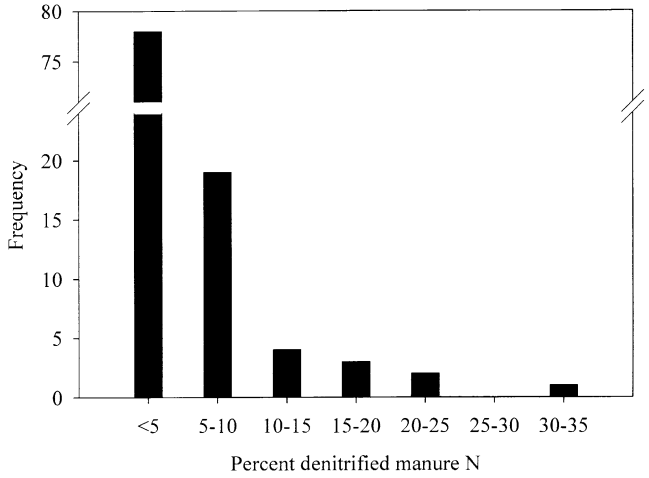

Fig. 4. Frequency distribution of the percentage denitrified manure N during the 6-wk incubation. n = 107. The values were calculated as (total denitrified N-control denitrified N)/added manure N.

The multiple regression results show that the measured manure properties alone or in combination could only account for a small amount of the variation in manure mineralizable N. The stepwise regression achieved a maximum R^2 score of 0.42 after 10 steps (Table 6). Similarly, the Forward Selection, Maximum R^2 improvement, and Full Model Fitted methods did not achieve an R^2 better than 0.42 (data not shown).

DISCUSSION

Denitrification and Nitrogen Mineralization

In this study, denitrification resulted in average N losses that amounted to <5% of the added manure N, but about 30% of the added NH_4–N. This is a significant amount of N, considering that not all manure organic N is readily available to microbes. The cumulative denitrified N and negative N mineralization amounted to similar absolute values at about 4.9 mg kg^{-1}. However, including the denitrified N as part of the net mineralizable N did not improve the correlation of mineralizable N with added manure N. Some manures resulted in denitrification rates that amounted to higher than 10% of the added manure N. Because of this, we hypothesize that manure N mineralization potential incubation assays may result in underestimates, unless denitrification is quantified. This problem could be exacerbated when manure is applied to soils with restricted aerobicity such as very moist soils or soils with high bulk density.

While N_2O fluxes were low at the end of the incubation, the DEA assay showed that enzyme concentrations remained high in the manured soils until the end of the incubation. This indicates that the positive effect of manuring soil on potential denitrification continues for days and weeks after the high N_2O fluxes subside. The lack of correlation between DEA, net ammonification, net nitrification, and C variables may indicate that denitrification was not C or N limited in the manured soils. Alternatively, this lack of correlation may be caused by complex relationships between the many variables that preclude simple correlations. The control soils were

Table 5. N_2O-N production and CO_2–C production during the denitrification enzyme activity (DEA) assay measured at the end of the 6-wk incubation. n = 107 for the manured soil. n = 6 for the control soils.

Variable	Average (SEM)	
	Manured soils	Control soils
	mg kg^{-1} h^{-1}	
N_2O	0.34 [a] (0.01)†	0.24 [b] (0.02)
CO_2	2.08 [a] (0.09)	1.82 [a] (0.23)

† Means not sharing a letter within a row are statistically different according to ANOVA ($p < 0.01$).

characterized by very small N_2O fluxes as well as low DEA relative to the manure treatments. The positive correlation between initial soil nitrate and DEA suggests that denitrification was limited by nitrate availability in the control soils. The fact that acetylene did not stimulate N_2O production further supports that denitrification was negligible in the controls. However, the correlation between cumulative CO_2 and cumulative N_2On in the controls suggests a relationship between microbial activity and N oxide fluxes.

The observed N_2Oa, as well as the increased DEA in the manured soils indicates that denitrifiers were favored by manure addition. We hypothesize that the increased nitrate and assimilable C in the manured soil combined to produce anaerobic microsites with increased denitrification activity. The lack of correlation between N_2O fluxes and NO_3^- may be due to a rapid C and N cycling that did not allow pool sizes to represent nutrient availability. The combined low N_2O fluxes and high NO_3^- concentration during the last 3 wk of the incubation suggests that denitrifiers were C-limited during this time period.

Denitrification rates usually reach maximum values between water filled pore spaces of 60 to 100% (Groffman and Tiedje, 1988). In this study, average water filled pore space ranged from 23.1 to 25.5%. Because of this, we hypothesize that the denitrification rates reported in this study are moderate, and higher moisture contents may have resulted in proportionately higher denitrification N losses.

Manure Variables and Nitrogen mineralization

It is possible that measurement of more specific manure organic N fractions may have improved our understanding of the relationship between manure N content and manure mineralizable N. Our analysis of manure N pools was limited to total manure N, manure NH_4–N, and manure organic N. Other forms of manure N such as proteins or amino acids were not analyzed. Alternatively, the lack of correlation between manure N pools and manure N mineralization could have improved by including a measure of immobilized N. Because of this, future studies should include measurements of microbial biomass N throughout the incubation, as well as more specific manure N pools.

Including the lignin/N ratio as an independent variable may improve the estimate of the amount of mineralizable N (Vigil and Kissel, 1991). The ADF, NDF, and lignin in dairy manure are a result of undigested forage cell walls, with each fraction having different mineralization properties (Van Kessel et al., 2000; Van Kessel and Reeves, 2002). Our data shows that none of the fiber variables (ADF, NDF, or lignin) showed a strong correlation with C mineralization or N mineralization during the incubation, suggesting that these fractions contain relatively resistant C and do not significantly affect net nutrient immobilization or mineralization. Alternatively, it is possible that these manure components are only part of a multivariate set of parameters that take part in the mineralization kinetics and simple correlations will not illustrate their importance.

Beauchamp and Paul (1989) suggested that manures with C/N ratios below 15 are likely to result in positive N mineralization after application to soil. In this study, the C/N ratio does play a role in the mineralization characteristics of the manures, since manures associated with positive N mineralization had a mean C/N of 16.0, while manures associated with negative N mineralization had on average a C/N of 19.0.

Volatile fatty acids are part of the water-soluble manure C that is readily accessible to microbial utilization (Paul and Beauchamp, 1989b; Kirchmann and Lundvall, 1993). It has been shown that VFA serve as C sources for denitrifiers, leading to increased nitrogen oxide and CO_2 fluxes from manure-amended soils (Paul and Beauchamp, 1989a, 1989b). The VFA are important C sources and favor O_2 consumption by soil microbes, creating beneficial conditions for denitrifiers in recently manured soils (Paul and Beauchamp, 1989a, 1989b). However, in this study, the lack of correlation between VFA and N pools suggest that other sources besides VFA play a role as C sources for denitrifiers. The lack of correlation between manure VFA and net N mineralization contrast with the findings of Kirchmann and Lundvall (1993), who found a strong correlation of VFA and negative N mineralization after application of animal

Table 6. Summary of stepwise selection of the stepwise regression procedure. The analysis was performed using the STEPWISE Model-Selection of the PROC REG procedure of SAS version 8.2 (Cary, NC).

Step	Variable	Units	Partial R^2	Model R^2	C_p	F value	Pr. > F
1	isobutyric acid	mg g^{-1}	0.10	0.10	85.05	24.00	<0.0001
2	manure H_2O content	%	0.05	0.15	72.09	11.23	0.001
3	manure NH_4–N	mg g^{-1}	0.08	0.23	47.78	21.72	<0.0001
4	manure N/N ratio		0.08	0.31	24.02	23.58	<0.0001
5	propionic acid	mg g^{-1}	0.03	0.34	15.34	10.22	0.002
6	lignin	%	0.02	0.36	11.64	5.57	0.019
7	isovaleric acid	mg g^{-1}	0.02	0.37	8.76	4.85	0.029
8	acid detergent fiber	%	0.01	0.39	6.19	4.63	0.033
9	manure organic N	%	0.01	0.40	4.75	3.53	0.062
10	manure total C	%	0.01	0.41	4.04	2.82	0.095

manure to soil. Our results suggest that VFA were not associated with declines in soil mineral N, since some of the soils with the highest negative mineralization values were also low in added VFA. It is possible that a continuous measurement of VFA concentration during the incubation might have been necessary to elucidate the role of VFA on net N mineralization. The weak but significant correlation between manure VFA and manure ammonium might have arisen due to anaerobic conditions during storage in the field previous to the experiment (Paul and Beauchamp, 1989b).

Soil Variables and Nitrogen Mineralization

Manure supplied assimilable C to the soil and resulted in a high flux of CO_2 relative to the control soil. Robertson et al. (1988) proposed the use of C mineralization as an index of soil mineralizable N. Other authors have also proposed that short-term CO_2 production after manure addition as a reliable index of net mineralizable N (Haney et al., 2001). In contrast, we did not find a correlation between CO_2 flux and any of the mineral N pools during the 6-wk incubation. However, high CO_2 fluxes as well as N mineralization may have occurred within the first week of the incubation, so a finer sampling schedule at the beginning of the incubation might have shown a closer relationship between mineralizable N and CO_2 flux.

Manure addition increased the soil mineral N content throughout the incubation, despite the negative mineralizable N values. In the manured soils, increased denitrification does not fully account for the negative net mineralizable N. Previous studies of manure incubation in closed systems have shown that losses of soil N through ammonia volatilization are negligible (Kirchmann and Lundvall, 1993). We hypothesize that N immobilization contributed to the reduction in mineral N during the incubation. Manure C fueled the apparent immobilization of initial NH_4^+ supplied by the manure.

It has been shown that soil microbial biomass has a preference of NH_4^+ rather than NO_3 as a mineral N source (Rice and Tiedje, 1989). In this study, the negative N mineralization associated with many of the manures suggests that the incubation conditions, as well as the assimilable C in the manures favored the immobilization of NH_4^+ by the soil microbiota. We observed net nitrification in the manured and control soils during the incubation, indicating that nitrifiers were important sinks for NH_4^+. The lag in NH_4^+ and NO_3 changes between Week 0 and 1 may be explained by the acetylene blockage of nitrification in the microcosms sampled at Week 1. Net nitrate consumption in the manured soil during the first week may be due to utilization by denitrifiers concurrent with blocked nitrification. After Week 1, all jars had periods of incubation without acetylene blockage, allowing for nitrification to take place. Nitrification is a strictly aerobic process, thus, the nitrate accumulation in the microcosms suggests that the soils had adequate O_2 supply during the incubation. It is possible that longer incubations might have yielded positive mineralizable N values in the manured soil. However, this study suggests that application of manure may result in short-term declines in N availability in agricultural soil. Temporary declines in soil mineral N after manure addition have been observed by others (Kirchmann and Lundvall, 1993; Serna and Pomares, 1991).

The mineralizable C/mineralizable N ratio has been used as an index of organic matter in soil (Carter and Rennie, 1982; Robertson et al., 1988). In this experiment, the negative N mineralization observed with most of the manures indicates that simply quantifying the net mineralizable C/mineralizable N ratio is not sufficient to understand the value of manure as an N fertilizer. The N cycling in the manure-amended soils was dominated by the high initial concentration of NH_4^+. This resulted in high net nitrification and apparent immobilization during the incubation.

Other studies have shown that application of dairy slurries increase soil N_2O fluxes (Comfort et al., 1990; Barton and Schipper, 2001). Similarly, in this study, manured soils had significantly higher N_2O fluxes relative to control soils. Labile N and C indirectly determine the quantity of nitrogen oxides entering the atmosphere (Weier et al., 1993). However, our results show no correlation of cumulative N_2O flux with mineralizable N, manure C/N, or any of the N pools measured during the experiment. It is possible that the fixed amount of manure N added to the microcosms resulted in a relatively limited range of mineralized N and N oxide fluxes, precluding a correlation of variables.

CONCLUSIONS

This experiment shows that measurement of mineral N alone may not be adequate for estimates of mineralizable N in manured soils. Added manure N and C favor denitrification in otherwise aerobic microcosms, leading to losses of mineralized N. We recognize that N lost as N_2 or N_2O from manured soils may have originated from manure mineralized N and could reasonably be part of the calculations of manure net N mineralization. However, no consensus exists yet as to what should be done with the sometimes-large denitrification N loss during manure incubation. Nitrogen losses through denitrification made up a significant portion of the added manure N, suggesting that under specific conditions, denitrification will need to be monitored to make correct estimates about a manure's ability to supply N. We propose that the quantification of mineralizable N in manured soils should include measurements of microbial biomass N, N_2Oa, or alternatively, use of nitrification inhibitors to curtail the nitrate supply to denitrifiers during the incubation. The apparent N immobilization during this experiment suggests that manure addition is not only an important source of immediately available N, but also that remineralization of immobilized N may be an important source of N beyond the 6-wk period used for this experiment.

ACKNOWLEDGMENTS

We thank Richard Brown, Barry Francis, Swati Mookherji, and Robert Ransom for all the help given during the sampling and analyses.

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